US00RE47301E

(19) **United States**

(12) **Reissued Patent**

Zablocki et al.

(10) **Patent Number: US RE47,301 E**

(45) **Date of Reissued Patent: Mar. 19, 2019**

(54) **PROCESS FOR PREPARING AN A2A-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS**

(71) Applicant: **Gilead Sciences, Inc.**, Foster City, CA (US)

(72) Inventors: **Jeff Zablocki**, Los Altos, CA (US); **Elfatih Elzein**, Mountain House, CA (US); **Robert Seemayer**, Belmont, CA (US); **Travis Lemons**, Los Gatos, CA (US)

(73) Assignee: **Gilead Sciences, Inc.**, Foster City, CA (US)

(21) Appl. No.: **15/653,860**

(22) Filed: **Jul. 19, 2017**

Related U.S. Patent Documents

Reissue of:

(64) Patent No.: **9,085,601**
Issued: **Jul. 21, 2015**
Appl. No.: **13/970,372**
Filed: **Aug. 19, 2013**

U.S. Applications:

(63) Continuation of application No. 13/333,872, filed on Dec. 21, 2011, now Pat. No. 8,524,833, which is a continuation of application No. 12/765,623, filed on Apr. 22, 2010, now Pat. No. 8,106,183, which is a continuation of application No. 11/701,699, filed on Feb. 2, 2007, now Pat. No. 7,732,595.

(60) Provisional application No. 60/801,857, filed on May 18, 2006, provisional application No. 60/765,114, filed on Feb. 3, 2006.

(51) **Int. Cl.**

A01N 43/04	(2006.01)
A61K 31/70	(2006.01)
C07H 19/00	(2006.01)
C07H 19/167	(2006.01)
C07H 19/173	(2006.01)
C07H 19/16	(2006.01)

(52) **U.S. Cl.**

CPC ***C07H 19/16*** (2013.01); ***C07H 19/167*** (2013.01)

(58) **Field of Classification Search**

CPC A01N 43/04; A61K 31/70; C07H 19/00; C07H 19/167; C07H 19/173; C07H 19/16

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,845,770	A	11/1974	Theeuwes et al.
4,089,959	A	5/1978	Diamond
4,120,947	A	10/1978	Diamond
4,325,956	A	4/1982	Kjellin et al.
4,326,525	A	4/1982	Swanson et al.
4,593,095	A	6/1986	Snyder et al.
4,696,932	A	9/1987	Jacobson et al.
4,804,664	A	2/1989	Kjellin et al.
4,902,514	A	2/1990	Barclay et al.
4,956,345	A	9/1990	Miyasaka et al.
4,968,687	A	11/1990	Findeisen et al.
4,968,697	A	11/1990	Hutchison
4,990,498	A	2/1991	Suhadolnik
4,992,445	A	2/1991	Lawter et al.
5,001,139	A	3/1991	Lawter et al.
5,032,252	A	7/1991	Owen et al.
5,070,877	A	12/1991	Mohiuddin et al.
5,189,027	A	2/1993	Miyashita et al.
5,270,304	A	12/1993	Kogi et al.
5,459,254	A	10/1995	Yamaguchi et al.
5,477,857	A	12/1995	McAfee et al.
5,516,894	A	5/1996	Reppert
5,593,975	A	1/1997	Cristalli
5,616,345	A	4/1997	Geoghegan et al.
5,641,784	A	6/1997	Kufner-Muhl et al.
5,646,156	A	7/1997	Jacobson et al.
5,670,498	A	9/1997	Suzuki et al.
5,703,085	A	12/1997	Suzuki et al.
5,704,491	A	1/1998	Graves
5,705,491	A	1/1998	Yamada
5,770,716	A	6/1998	Khan et al.
5,776,960	A	7/1998	Oppong et al.
5,780,481	A	7/1998	Jacobson et al.
5,854,081	A	12/1998	Linden et al.
5,877,180	A	3/1999	Linden et al.
5,939,543	A	8/1999	Morozumi et al.
6,026,317	A	2/2000	Verani
6,117,878	A	9/2000	Linden
6,214,807	B1	4/2001	Zablocki et al.
6,294,522	B1	9/2001	Zablocki et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	965411	4/1975
CA	2064742	12/1991

(Continued)

OTHER PUBLICATIONS

U.S. Appl. No. 10/896,766, filed Jul. 22, 2004, Vanderbilt University.

U.S. Appl. No. 12/637,311, filed Dec. 14, 2009, Zablocki et al.

U.S. Appl. No. 14/701,285, filed Apr. 30, 2015, Zablocki et al.

Jeffery, "Remodeling in Asthma and Chronic Obstructive Lung Disease," Am. J. Respir. Crit. Care Med., vol. 164, No. 10pt2, pp. S28-S38 (2001).

Office Action for U.S. Appl. No. 13/092,812, dated Oct. 25, 2011 (citing Brady et al., "General Chemistry—Principles and Structure", John Wiley & Sons, New York, 1978, pp. 328-330 and 352-353).

Avis, Kenneth E., Sterile Products, *The Theory and Practice of Industrial Pharmacy* edited by Lachman, Leon et al., Fourth Indian Reprint, 1991, pp. 639-677, Varghese Publishing House, Bombay, India.

(Continued)

Primary Examiner — Jerry D Johnson

(74) *Attorney, Agent, or Firm* — Sheppard Mullin Richter & Hampton LLP

(57) **ABSTRACT**

Disclosed is a synthesis suitable for large scale manufacture of an A_{2A}-adenosine receptor agonist, and also relates to polymorphs of that compound, and to methods of isolating a specific polymorph.

12 Claims, 5 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

6,322,771	B1	11/2001	Linden et al.	
6,368,573	B1	4/2002	Leung	
6,387,913	B1	5/2002	Mustafa	
6,403,567	B1 *	6/2002	Zablocki et al.	514/46
6,448,235	B1	9/2002	Linden et al.	
6,514,949	B1	2/2003	Linden et al.	
6,552,023	B2	4/2003	Zablocki et al.	
6,599,283	B1	7/2003	Marzilli et al.	
6,605,597	B1	8/2003	Zablocki et al.	
6,642,210	B1 *	11/2003	Zablocki et al.	514/46
6,670,334	B2	12/2003	Linden et al.	
6,677,336	B2	1/2004	Zablocki et al.	
6,758,388	B1	7/2004	Leholm et al.	
6,770,634	B1	8/2004	Zablocki et al.	
6,825,349	B2	11/2004	Kalla et al.	
6,855,818	B2	2/2005	Zablocki et al.	
6,916,804	B2	7/2005	Castelhano et al.	
6,977,300	B2	12/2005	Kalla et al.	
6,995,148	B2	2/2006	Jones et al.	
7,109,180	B2	9/2006	Zablocki et al.	
7,109,203	B2	9/2006	Hart et al.	
7,125,993	B2	10/2006	Elzein et al.	
7,144,872	B2	12/2006	Zablocki et al.	
7,183,264	B2 *	2/2007	Zablocki et al.	514/46
7,553,823	B2	6/2009	Zablocki et al.	
7,582,617	B2	9/2009	Belardinelli et al.	
7,655,636	B2	2/2010	Gordi et al.	
7,655,637	B2	2/2010	Zablocki et al.	
7,671,192	B2 *	3/2010	Zablocki et al.	536/27.11
7,683,037	B2	3/2010	Belardinelli	
7,732,595	B2 *	6/2010	Zablocki et al.	536/27.11
7,956,179	B2 *	6/2011	Zablocki et al.	536/27.11
8,106,029	B2	1/2012	Gordi et al.	
8,106,183	B2 *	1/2012	Zablocki et al.	536/27.11
8,133,879	B2	3/2012	Belardinelli et al.	
8,183,226	B2	5/2012	Belardinelli et al.	
8,268,988	B2	9/2012	Zablocki et al.	
8,278,435	B2	10/2012	Zablocki et al.	
8,470,801	B2	6/2013	Belardinelli et al.	
8,524,883	B2 *	9/2013	Zablocki et al.	536/27.11
8,536,150	B2	9/2013	Belardinelli et al.	
8,569,260	B2	10/2013	Zablocki et al.	
9,045,519	B2	6/2015	Zablocki et al.	
9,289,446	B2	3/2016	Belardinelli et al.	
9,441,006	B2 *	9/2016	Kvapil	C07H 19/16
9,580,457	B2 *	2/2017	Pullagurla	C07H 1/00
9,624,258	B2 *	4/2017	Rangisetty	C07H 19/16
9,771,390	B2 *	9/2017	Zhang	C07H 19/167
2002/0012946	A1	1/2002	Belardinelli et al.	
2002/0111327	A1	8/2002	Linden et al.	
2002/0147174	A1	10/2002	Jones et al.	
2003/0235555	A1	12/2003	Shealey et al.	
2004/0038928	A1	2/2004	Zablocki et al.	
2004/0064039	A1	4/2004	Belardinelli	
2004/0127533	A1	7/2004	Hart et al.	
2004/0137533	A1	7/2004	Belardinelli et al.	
2005/0020915	A1	1/2005	Belardinelli et al.	
2005/0175535	A1	8/2005	Belardinelli et al.	
2006/0084625	A1	4/2006	Gordi et al.	
2006/0159621	A1	7/2006	Barrett	
2006/0159627	A1	7/2006	Zeng et al.	
2007/0114269	A1	5/2007	Straza	
2007/0203090	A1	8/2007	Zablocki et al.	
2007/0207978	A1	9/2007	Zablocki et al.	
2007/0265445	A1	11/2007	Zablocki et al.	
2007/0299089	A1	12/2007	Belardinelli et al.	
2008/0170990	A1	7/2008	Lieu et al.	
2008/0213165	A1	9/2008	Lieu et al.	
2008/0267861	A1	10/2008	Lieu et al.	
2009/0081120	A1	3/2009	Lieu et al.	
2009/0317331	A1	12/2009	Belardinelli et al.	
2010/0081810	A1	4/2010	Zablocki et al.	
2010/0086483	A1	4/2010	Belardinelli et al.	
2010/0160620	A1	6/2010	Zablocki et al.	

FOREIGN PATENT DOCUMENTS

CA	2377746		12/2000
CA	2640089		8/2007
CN	1358191	A	7/2002
EP	0 354 638		2/1990
EP	354 638	A2 *	2/1990
EP	0 386 683		9/1990
EP	2 581 381		4/2013
JP	S 4826038		8/1973
JP	SHO 48 1973 26038		8/1973
JP	Hei 5 1993 9197		1/1993
WO	WO 1992/00297		1/1992
WO	WO 1992/12260		7/1992
WO	WO 93/23401		11/1993
WO	WO 93/25677		11/1993
WO	WO 1993/23401		11/1993
WO	WO 1993/25677		12/1993
WO	WO 1995/11681		5/1995
WO	WO 1998/052611		11/1998
WO	WO 1998/57651		12/1998
WO	WO 1999/63938		12/1999
WO	WO 2000/078778		12/2000
WO	WO 2000/078779		12/2000
WO	WO 2001/016134		8/2001
WO	WO 2001/062979		8/2001
WO	WO 2003/088978		10/2003
WO	WO 2004/011010		2/2004
WO	WO 2005/082379		9/2005
WO	WO 2006/044856		4/2006
WO	WO 2006/076698		7/2006
WO	WO 2007/092372		8/2007
WO	WO 2008/028140		3/2008
WO	WO 2008/042796		4/2008
WO	WO 2008/063712		5/2008
WO	WO 2008/086096		7/2008
WO	WO 2008/143667		11/2008
WO	WO 2006/044856		4/2009
WO	WO 2009/076580		6/2009
WO	WO 2010/037122		4/2010

OTHER PUBLICATIONS

Brittain, Harry G. et al., Effects of Pharmaceutical Processing on Drug Polymorphs and Solvates, *Polymorphism in Pharmaceutical Solids* edited by Brittain, Harry G., 1999, pp. 331-361, Marcel Dekker, Inc., New York, NY.

Brittain, Harry G. et al., Effects of Polymorphism and Solid-State Solvation on Solubility and Dissolution Rate, *Polymorphism in Pharmaceutical Solids* edited by Brittain, Harry G., 1999, pp. 279-330, Marcel Dekker, Inc., New York, NY.

Brittain, Harry G., Methods for the Characterization of Polymorphs and Solvates, *Polymorphism in Pharmaceutical Solids* edited by Brittain, Harry G., 1999, pp. 227-278, Marcel Dekker, Inc., New York, NY.

Guillory, J. Keith, Generation of Polymorphs, Hydrates, Solvates, and Amorphous Solids, *Polymorphism in Pharmaceutical Solids* edited by Brittain, Harry G, 1999, pp. 183-226, Marcel Dekker, Inc., New York, NY.

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances Q6A, Oct. 6, 1999, 35 pages.

U.S. Appl. No. 13/591,008, filed Aug. 21, 2012, Zablocki et al.

Bergmann et al., "Oxidation of Hypoxanthines, Bearing 8-Aryl or 8-Pyridyl Substituents, byBovine Milk Xanthine Oxidase,", Biochimica et Biophysica Acta, vol. 484, No. 2, pp. 275-289 (1977).

Birdsall et al., "Purine N-Oxides-XL the 3-Acyloxypurine 8-Substitution Reaction: Scope:Syntheses of 8-Substituted Xanthines and Guanines," Tetrahedron, vol. 27, pp. 5969-5978 (1971).

Blackburn et al., "Adenosine Mediates IL-13-Induced Inflammation and Remodeling in the Lung and interacts in an IL 13-Adenosine Amplification Pathway," J. Clin. Invest., vol. 112, No. 3, pp. 332-344 (2003).

(56) **References Cited**

OTHER PUBLICATIONS

Brady et al., "General Chemistry—Principles and Structure", John Wiley & Sons, New York, 1978, pp. 328-330 and 352-353.
Bruns, "Adenosine Antagonism by Purines, Pteridines and Benzopteridines in Human Fibroblasts," Biochemical Pharmacology, vol. 30, No. 4, pp. 325-333 (1981).
Buckle et al., "Inhibition of Cyclic Nucleotide Phosphodiesterase by Derivatives of 1,3-Bis(cyclopropylmethyl)xanthine," J. Med. Chem., vol. 37, pp. 476-485 (1994).
Caira, "Crystalline Polymorphism of Organic Compounds," Topics in Current Chemistry, Berlin, 1998, pp. 163-208.
Cerqueira, "The Future of Pharmacologic Stress: Selective A2A Adenosine Receptor Agonists," Am. J. Cardiol., vol. 94 (2A), pp. 33D-42D (2004).
Cline et al., "Coronary Artery Angiography Using Multislice Computed Tomography Images," Circulation, vol. 102, pp. 1589-1590, XP002564059 (2000).
Crimi et al., "Purine Derivatives in the Study of Allergic Inflammation in Respiratory Diseases," Allergy, vol. 52, No. 34, pp. 48-54 (1997).
Cristalli et al., "2-Alkynyl Derivatives of Adenosine 5'-N'ethyluronamide: Selective A2 Adenosine Receptor Agonists with Potent Inhibitory Activity on Platelet Aggretation," J. Med. Chem., vol. 37, pp. 1720-1726 (1994).
Cushley et al., "Inhaled Adenosine and Guanosine on Airway Resistance in Normal and Asthmatic Subjects," Br. J. Clin. Pharmacol, vol. 15, No. 2, pp. 161-165 (1983).
Dalpiaz et al., "De Novo Analysis of Receptor Binding Affinity Data of Xanthine AdenosineReceptor Antagonists," Arzneim-Forsch/Drug Res., vol. 45, No. 3, pp. 230-233 (1995).
Dhalla et al., "Tachycardia Caused by A2A Adenosine Receptor Agonists is Mediated by Direct Sympathoexcitation in Awake Rates," Journal of Pharmacology and Experimental Therapeutics, USA, vol. 316, No. 2, pp. 695-702, XP009073100 (2006).
Driver et al., "Adenosine in Bronchoalveolar Lavage Fluid in Asthma," Am. Rev. Respir. Dis., vol. 148, No. 1, pp. 91-97 (1993).
Elias et al., "Airway Remodeling in Asthma," The Journal of Clinical Investigation, vol. 104, No. 8, pp. 1001-1006 (1999).
Erickson et al., "1,3,8-Trisubstituted Xanthines. Effects of Substitution Pattern upon AdenosineReceptor A1/A2 Affinity", J. Med. Chem., vol. 34, pp. 1431-1435 (1991).
Feoktistov et al., "Adenosine A2B Receptors: A Novel Therapeutic Target in Asthma," Trends Pharmacol. Sci., vol. 19, pp. 148-153 (1998).
Feoktistov et al., "Hypoxia Modulates Adenosine Receptors in Human Endothelial and Smooth Muscle Cells Toward an A2B Angiogenic Phenotype," Hypertension, vol. 44, No. 5, pp. 649-654, Epub 2004, PMID: 15452028 [PubMed—indexed for MEDLINE] (2004).
Gao et al., "Novel Short-Acting A2A Adenosine Receptor Agonists for Coronary Vasodilation: Inverse Relationship between Affinity and Duration of Action of A2A Agonists," Journal of Pharmacology and Experimental Therapeutics, vol. 298, pp. 209-218 (2001).
Glover et al., "Characterization of a New, Highly Selective Adenosine A2A Receptor Agonist with Potential Use in Pharmacologic Stress Perfusion Imaging", Circulation, vol. 110, pp. I-311 (1999).
Glover et al., "Pharmacological Stress Thallium Scintigraphy with 2-cyclohexylmethylidenehydrazinoadenosine (WRC-0470)," Circulation, vol. 94, pp. 1726-1732 (1996).
Harvey, "Blood Fluids, Electrolytes and Hematologic Drugs," Chapter 40 in Remington's Pharmaceutical Sciences, 18th Ed., Gennaro et al., Mack Publishing Co., East, PA, only pp. 800 and 821 (1990).
Hasenfuss, "Animal Models of Human Cardiovascular Disease, Heart Failure and Hypertrophy", Cardiovascular Res., vol. 39, No. 1, pp. 60-76 (1998).
Hendel et al. "Initial Clinical Experience with Regadenoson, a Novel Selective A2A Agonist for Pharmacologic Stress Single-Photon Emission Computed Tomography Myocardial Perfusion Imaging", Journal of the American College of Cardiology, vol. 46, No. 11, pp. 2069-2075 (2005).
Hendel et al., "Pharmacologic Stress SPECT Myocardial Perfusion Imaging with a Selective A2A Agonist: Results of a Pilot Study Comparing Adenosine with CVT-3146", Circulation, Supplement IV, vol. 108, p. 2892 (2003).
Holgate et al., "Roles of Cysteinyl Leukotrienes in Airway Inflammation, Smooth Muscle Function and Remodeling," J. Allergy Clin. Imunol. (Suppl):S18-34; discussion S34-6, Review, PMID:12532084 [PubMed—indexed for MEDLINE] (2003).
Hoshino, "Impact of Inhaled Corticosteriods and Leukotrience Receptor Antagonists on Airway Remodeling," Clinical Reviews in Allergy & Immunology, vol. 27, No. 1, pp. 59-64 (2004).
Iskandrian, "Adenosine Myocardial Perfusion Imaging," The Journal of Nuclear Medicine, vol. 35, pp. 734-736 (1994).
Jacobson et al., "1,3-Dialkylxanthine Derivatives Having High Potency as Antagonists at HumanA2B Adenosine Receptors," Drug Development Research, vol. 47, pp. 45-53 (1999).
Jadbabaie et al., "Myocardial perfusion imaging with a novel selective A2A Adenosine Receptor Agonists (CVT-3146): Important differences in radiotracer behavior," Journal of Am. Col. Cardiology, vol. 41, pp. 443-444 (2003).
Katsushima et al., "Structure-Activity Relationships of 8-Cycloalkyl-1,3-dipropylxanthines asAntagonist of Adenosine Receptors," J. Med. Chem., vol. 33, pp. 1906-1910 (1990).
Kerensky et al. "Dose Dependent Increase in Human Coronary Blood Flow Velocity Following an IV Bolus of CVT-3146, a Novel A2A Adenosine Receptor Agonists: A Potential Agent for the Use in Pharmacological Stress Testing for Myocardial Perfusion Imaging," Circulation, Supplemental II, vol. 106, No. 19, pp. 11-618 (2002).
Kim et al., "Acyl-Hydrazide Derivatives of a Xanthine Carboxylic Congener (XCC) as Selective Antagonists at Human A2B Adenosine Receptors," Drug Development Research, vol. 47, pp. 178-188 (1999).
Kleiner, "Reactions of Some 8-(3-Pyridyl)-6-thioxanthines with Methyl Iodide," pp. 739-743 (1973).
Klotz et al., "Comparative pharmacology of human adenosine receptors subtypes-characterization of stably transfected receptors in CHO cells," Nauny-Schmideberg's Arch Pharmacol., vol. 357, pp. 1-9 (1998).
Knuniants et al., "Soviet Encyclopedia," Chemical Encyclopedia, Moscow, vol. 2, pp. 126-127, 1990 (translation).
Koepfli et al., "Interaction of caffeine with regadenoson-induced hyperemic myocardial blood flow as measured by PET," European Heart Journal, vol. 27, No. Supp. 1, p. 175 (2006).
Korolkovas, Essentials of Molecular Pharmacology-Background for Drug Design, Wiley—Interscience, New York, New York, only pp. 266-272 supplied, (1970).
Kubo et al., "Effect of Caffeine Intake on Myocardial Hyperemic Flow Induced by Adenosine Triphosphate and Dipyridamole," The Journal of Nuclear Medicine, vol. 45, No. 5, pp. 730-738, (2004).
Kusmic et al., "Coronary microcirculatory vasoconstriction induced by low-flow ischemia in mouse hearts is reversed by an A2A adenosine receptor", FASEB Journal, pp. A1227-A1228 (2007).
Leigh et al., "Is Interleukin-1 3 Critical in Maintaining Airway Hyperresponsiveness in Allergen-Challenged Mice?" Am. J. Respir. Crit. Care Med., PMID: 15242841 [PubMed—indexed for MEDLINE] vol. 170, No. 8, pp. 851-856 (2004).
Linden et al., "Characterization of Human A2B Adenosine Receptors: Radioligand Binding, WesternBlotting and Coupling to Gq in Human Embryonic Kidney 293 Cells and HMC-1 Mast Cells," Molecular Pharmacology, vol. 56, pp. 705-713 (1999).
Mager et al., "Molecular Simulation Applied to 2-(N'-alkylidenehydrazino)-and 2-(N'-aralkylidenehydrazino) adenosine A2 Agonists," European Journal of Medicinal Chemistry, vol. 30, pp. 15-25 (1995).
Mann et al., "Airway Effects of Purine Nucleosides and Nucleotides and Release With Bronchial Provocation in Asthma," J. Appl. Physiol., vol. 61, No. 5, pp. 1667-1676 (1986).
Martin et al., "Pharmacology of 2-cylohexylmethylidenehydrazionoadenosine (WRC-0470), a novel, short-acting adenosine A-2A receptor agonist that produces selective coronary vasodilation", Drug Development Research, vol. 40, No. 4, pp. 313-324, (1997).

(56) **References Cited**

OTHER PUBLICATIONS

Martinson et al., "Potent Adenosine Receptor Antagonists that are Selective for the A1 Receptor Subtype," Molecular Pharmacology, vol. 31, No. 3, pp. 247-252 (1986).

Marumoto et al., "Synthesis and Coronary Vasodilating Activity of 2-Substituted Adenosines," Chemical Pharmaceutical Bulletin, vol. 23, No. 4, pp. 759-774 (1975).

Marumoto et al., "Synthesis and Enzymatic Activity of Adenosine 3',5'-Cyclic Phosphate Analogues," Chemical Pharmaceutical Bulletin, vol. 27, No. 4, pp. 990-1003 (1979).

Matsuda et al., "Nucleosides and Nucleotides, 103. 2-Alkynyladenoines: A Novel Class of Selective Adenosine A2 Receptor Agonists with Potent Antihypertensive Effects," Journal of Medicinal Chemistry, vol. 35, No. 1, pp. 241-252 (1992).

Mosselhi et al., "Reactions of some 8-diazoxanthine derivatives," Indian Journal of Chemistry, vol. 33B, pp. 236-242 (1994).

Niiya et al., "2-(N'-Alkylidenehydrazino) Adenosines; Potent and Selective Coronary vasodilators," Journal of Medicinal Chemistry, American Chemical Society, vol. 35, No. 24, pp. 4557-4561, (1992).

Office Action for U.S. Appl. No. 11/848,743, dated Jun. 20, 2012.

Office Action for U.S. Appl. No. 12/163,099, dated Jun. 20, 2012.

Office Action for U.S. Appl. No. 12/569,643, dated Aug. 25, 2011.

Office Action for U.S. Appl. No. 12/569,643, dated Mar. 20, 2012.

Office Action for U.S. Appl. No. 12/695,096, dated Oct. 5, 2011.

Office Action for U.S. Appl. No. 12/695,096, dated Apr. 14, 2011.

Office Action for U.S. Appl. No. 12/749,328, dated Jun. 8, 2011.

Office Action for U.S. Appl. No. 12/968,110, dated Aug. 3, 2011.

Office Action for U.S. Appl. No. 13/092,812, dated Oct. 25, 2011.

Office Action for U.S. Appl. No. 13/333,789, dated Jul. 3, 2012.

Office Action for U.S. Appl. No. 13/361,775, dated Jul. 25, 2012.

Office Action for U.S. Appl. No. 13/525,223, dated Aug. 13, 2012.

Office Action for U.S. Appl. No. 11/864,437, dated Mar. 29, 2011.

Office Action for U.S. Appl. No. 11/969,047, dated Mar. 17, 2011.

Office Action for U.S. Appl. No. 12/435,176, dated Apr. 15, 2011.

Office Action for U.S. Appl. No. 12/637,583, dated Apr. 4, 2011.

Office Action for U.S. Appl. No. 12/765,623, dated Mar. 8, 2011.

Office Action for U.S. Appl. No. 11/766,964, dated Apr. 7, 2011.

Ogden, et al., Mean Body Weight, Height, and Body Mass Index, United States 1960-2002, U.S. National Health and Nutrition Examination Survey, Advance Data No. 347, pp. 1-18 (2004).

Persson et al., "Synthesis and Antiviral Effects of 2-Heteroaryl Substituted Adenosine and 8-Heteroaryl Guanosine Derivatives," Bioorganic & Medicinal Chemistry, vol. 3, No. 10, pp. 1377-1382 (1995).

Pfizer, "Health info.," (2003), http://www.pfizer.be/English/What_we_do_/Health_info/COPD.htm.

Pifferi et al., "Montelukast and Airway Remodeling in Children with Chronic Persistent Asthma: An Open Study," Pediatric Allergy and Immunology, vol. 15, No. 5, pp. L472-473 (2004).

Polosa et al., "Evolving Concepts on the Value of Adenosine Hyperresponsiveness in Asthma and Chronic Obstructive Pulmonary Disease". Thorax, vol. 57, No. 7, pp. 649-654 (2002).

Polosa, "Adenosine-Receptor Subtypes: The Relevance to Adenosine-Mediated Responses in Asthma and Chronic Obstructive Pulmonary Disease," The European Respiratory Journal: Official Journal of the European Society for Clinical Respiratory Physiology., vol. 20, No. 2, pp. 488-496 (2002).

Riou et al., "Influence of propranolol, enalaprilat, verapamil, and caffeine on adenosine A(2A) receptor medicated coronary vasodilation," Journal of the American College of Cardiology, vol. 40, No. 9, pp. 1687-1690 (2002).

Roth et al., "8-Dicyclopropylmethyl)-1,3-dipropylxanthine: A Potent and Selective Adenosine A1 Antagonist with Renal Protective and Diuretic Activities," J. Med. Chem., vol. 34, No. 1, pp. 466-469 (1991).

Ryzhov et al., "Adenosine-Activated Mast Cells Induce IgE Synthesis by B Lymphocytes: An A2B-Mediated Process Involving the Cytokines IL-4 and IL-13 with Implications for Asthma," vol. 172, No. 12, pp. 7726-7733, PMID: 15187156 [PubMed—indexed for MEDLINE] (2004).

Sambuceti et al., Coronary Vasoconstriction During Myocardial Ischemia Induced by Rises in Metabolic Demand in Patients with Coronary Artery Disease, Circulation, vol. 95, (2652-2659) pp. 1-24 (1997).

Sambuceti et al., Interaction Between Coronary Artery Stenosis and Coronary Microcirculcation in Ishcemic Heart Disease, Z Kardiol, 2000; 89 Suppl 9:IX/126-31, abstract.

Shimada et al., "8-Polycycloalkyl-l,3-dipropylxanthines as Potent and Selective Antagonists forA1—Adenosine Receptors," J. Med. Chem., vol. 35, pp. 924-930 (1992).

Spicuzza et al., "Research Applications and Implications of Adenosine in Diseased Airways," Trends Pharmacol. Sci., vol. 24, No. 8, pp. 409-413, Review, PMID: 12915050 [Pubmed—indexed for MEDLINE] (2003).

Spicuzza et al., "The Role of Adenosine as a Novel Bronchoprovocant in Asthma," Curr. Opin. Allergy Clin. Immunol., vol. 3, No. 1, pp. 65-69 (2003).

Swinyard et al., "Pharmaceutical Necessities," Chapter 66 in Remington's Pharmaceutical Sciences, 18th Ed., Gennaro et al. (eds.), Mack Publishing Co, Easton, PA, only pp. 1318-1319 supplied (1990).

Tomita et al., Artificial Neural Network Approach for Selection of Susceptible single Nucleotide Polymorphisms and Construction of Prediction Model on Childhood allergic Asthma: BMC Bioinformatics, vol. 1, No. 5, p. 120, PMID: 15339344 [PubMed—indexed for MEDLINE] (2004).

Trochu et al., "Selective A2A Adenosine Receptor Agonist as a Coronary Vasodilator in Conscious Dogs: Potential for Use in Myocardial Perfusion Imaging," Journal of Cardiovascular, vol. 41, No. 1, pp. 132-139 (2003).

Udelson et al., "Randomized, Controlled Dose-Ranging Study of the Selective Adenosine A2A Receptor Agonist Binodenoson for Pharmacological Stress as an Adjunct to Myocardial Perfusion Imaging," Circulation, vol. 209, pp. 457-464 (2004).

Van Der Wenden et al., "Mapping the Xanthine C8-region of the adenosine A1 Receptor withComputer Graphics," European Journal of Pharmacology-Molecular Pharmacology Section, vol. 206, No. 1, pp. 315-323 (1991).

Xu et al., Coronary Vasodilation by a Short Acting, Low Affinity A2A Adenosine Receptor Agonist in Anesthetize Closed Chest Dogs: A Second Generation of Coronary Artery Pharmacologic Stressor, Circulation, vol. 102, No. 18, p. 3912 (2000).

Zablocki et al. 2-Substituted PI System Derivatives of Adenosine that are Coronary Vasodilators Acting via the A2A Adenosine Receptor, Nucleosides, Nucleotides and Nucleic Acids, vol. 20, No. 4-7, pp. 343-360 (2001).

Zhao et al., "Caffeine attenuates the duration of coronary vasodilation and changes in hemodynamics induced by regadenoson (CVT-3146), a novel adenosine A2A receptor agonist," Journal of Cardiovascular Pharmacology, Raven Press, New York, New York, vol. 49, No. 6, pp. 369-375, XP009094871 (2007).

Zhao et al., "Comparative Profile of Vasodilation by CVT-3146, a novel A2A receptor agonist and adenosine in conscious dogs," Journal of Pharm & Experimental Therapeutics, Journal of Pharm. & Experimental Therapeutics, vol. 41, pp. 182-189 (2003).

Zhao et al., "Effects of caffeine on coronary vasodilation and sinue tachycardia induced by Regadenoson, a novel adenosine A2A receptor agonist, in conscious dogs," European Heart Journal, vol. 27, No. Suppl. 1, p. 424 (2006).

Zhao et al., "Regadenoson, a novel pharmacologic stress agent for use in myocardial perfusion imaging, does not have a direct effect on the QT interval in conscious dogs," Journal of Cardio Vascular Pharmacology, pp. 467-473, vol. 52, No. 5, Lippincott Williams and Wilkins, USA, XP8117431 (2008).

Zhong et al., "Synergy Between A2B Adenosine Receptors and Hypoxia in Activating Human Lung Fibroblasts," American Journal of Respiratory Cell and Molecular Biology, vol. 32, No. 1, pp. 2-8 (2005).

Extended European Search Report, dated Oct. 2, 2013, in related European Patent Application No. 12 008 201.1-1452.

(56) **References Cited**

OTHER PUBLICATIONS

International Preliminary Report on Patentability and Written Opinion, dated Jul. 2, 2007, in related International Patent Application No. PCT/US2007/003022.
English Translation of Office Action in Mexican Application No. MX/a/2010/014060 dated Nov. 10, 2014.

* cited by examiner

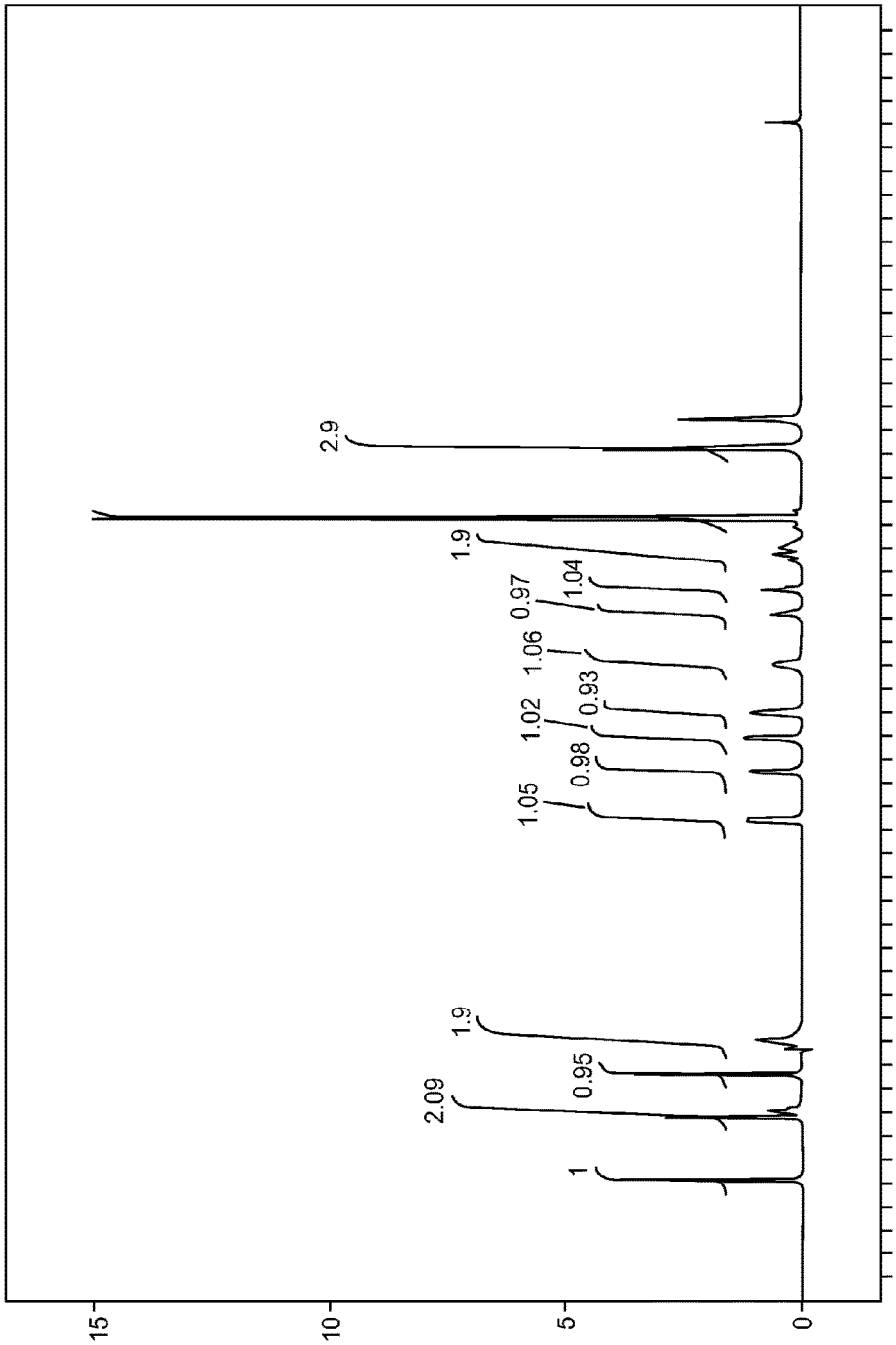

15
10
5
0
9.8
8.6
7.4
6.2
5
3.8
2.6
1.4
0.2
Parts per Million
2.9
1.9
0.97
1.04
1.06
0.93
1.02
0.98
1.05
1
2.09
0.95
1.9

FIG. 1

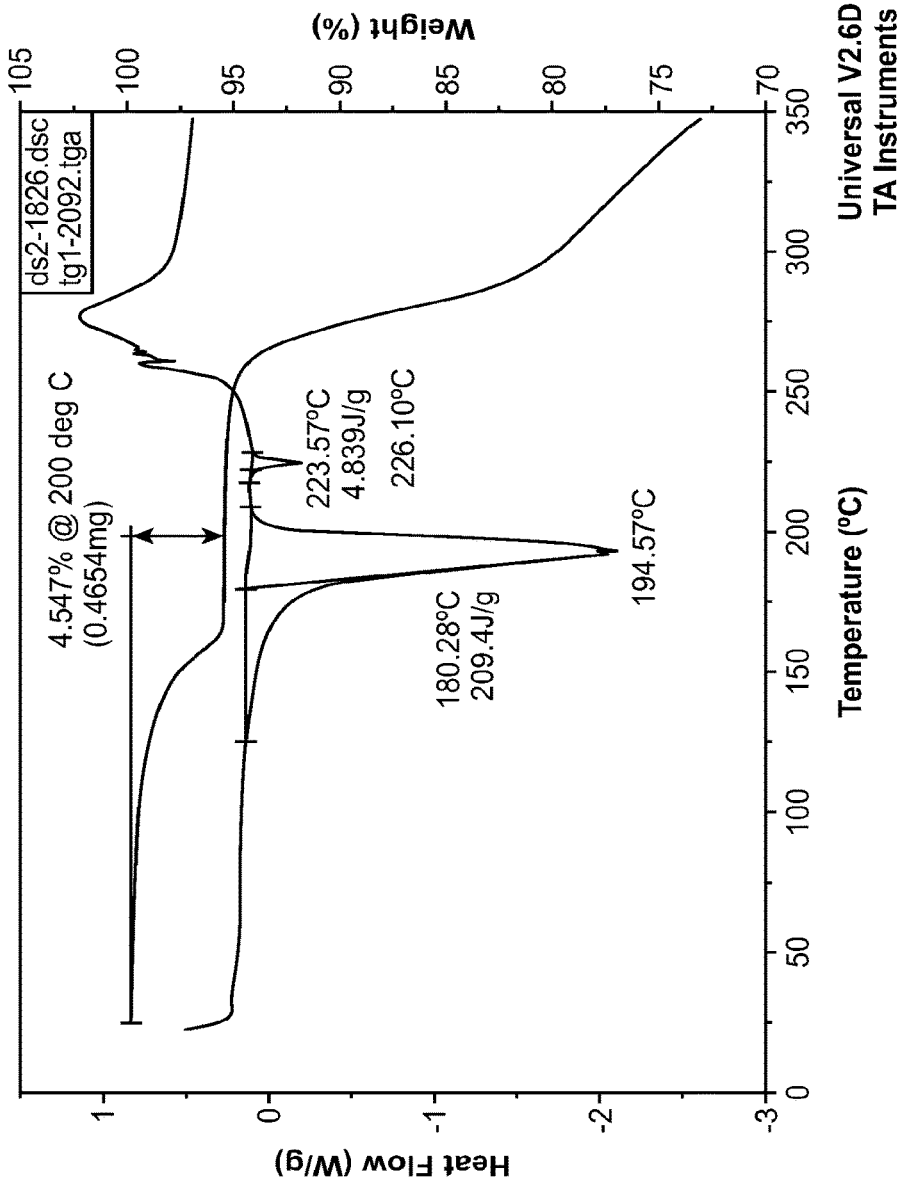

ds2-1826.dsc
tg1-2092.tga
4.547% @ 200 deg C
(0.4654mg)
223.57°C
4.839J/g
226.10°C
194.57°C
180.28°C
209.4J/g
Heat Flow (W/g)
Weight (%)
Temperature (°C)
Universal V2.6D
TA Instruments

FIG. 2

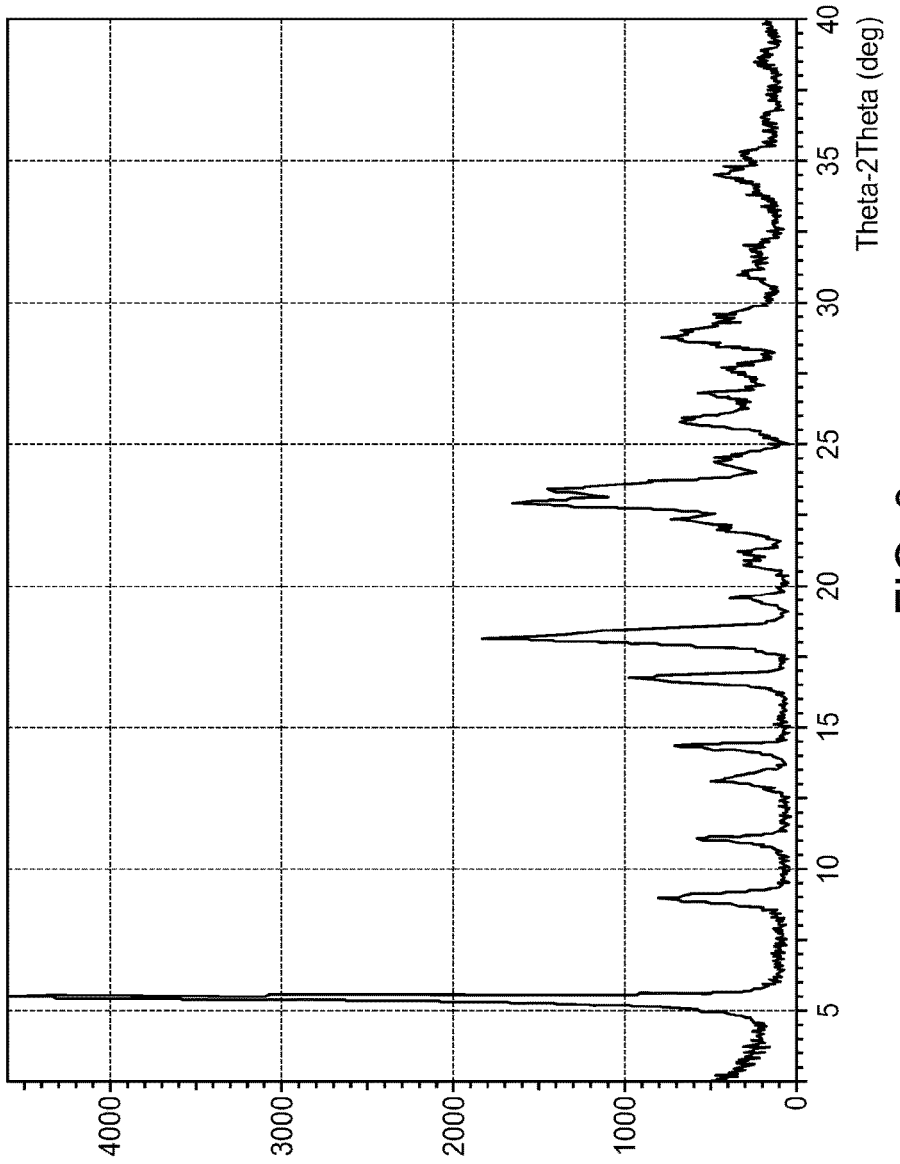


FIG. 3

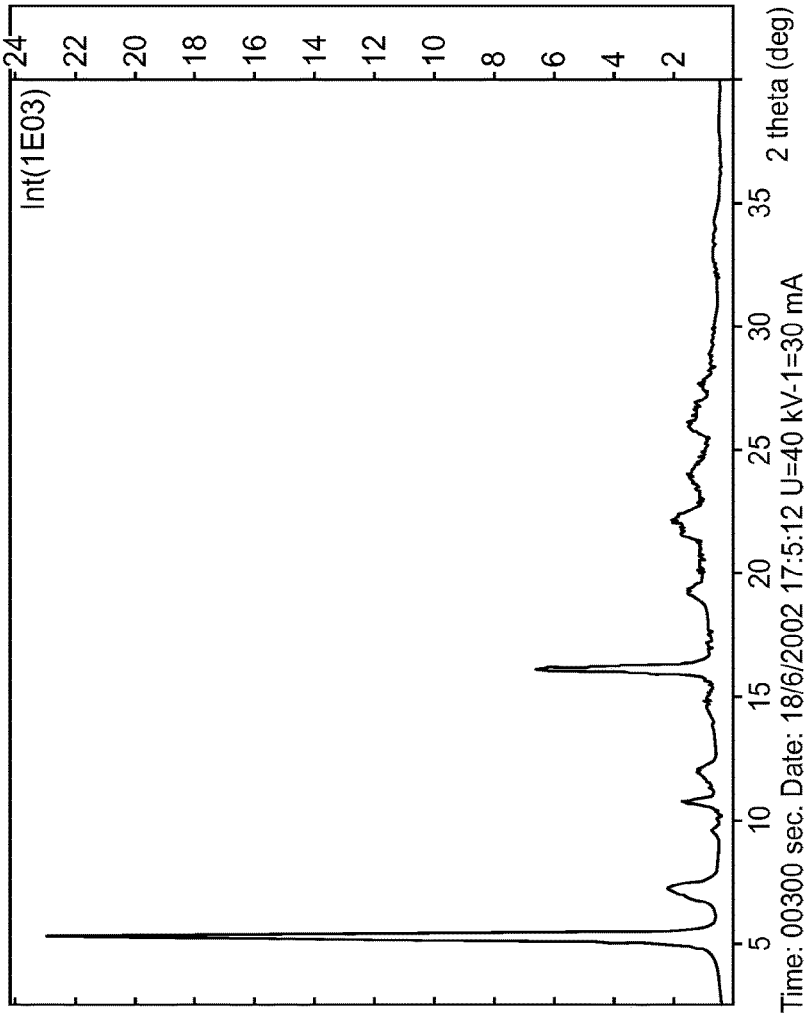

Int(1E03)
24
22
20
18
16
14
12
10
8
6
4
2
5
10
15
20
25
30
35
2 theta (deg)
Time: 00300 sec. Date: 18/6/2002 17:5:12 U=40 kV-1=30 mA

FIG. 4

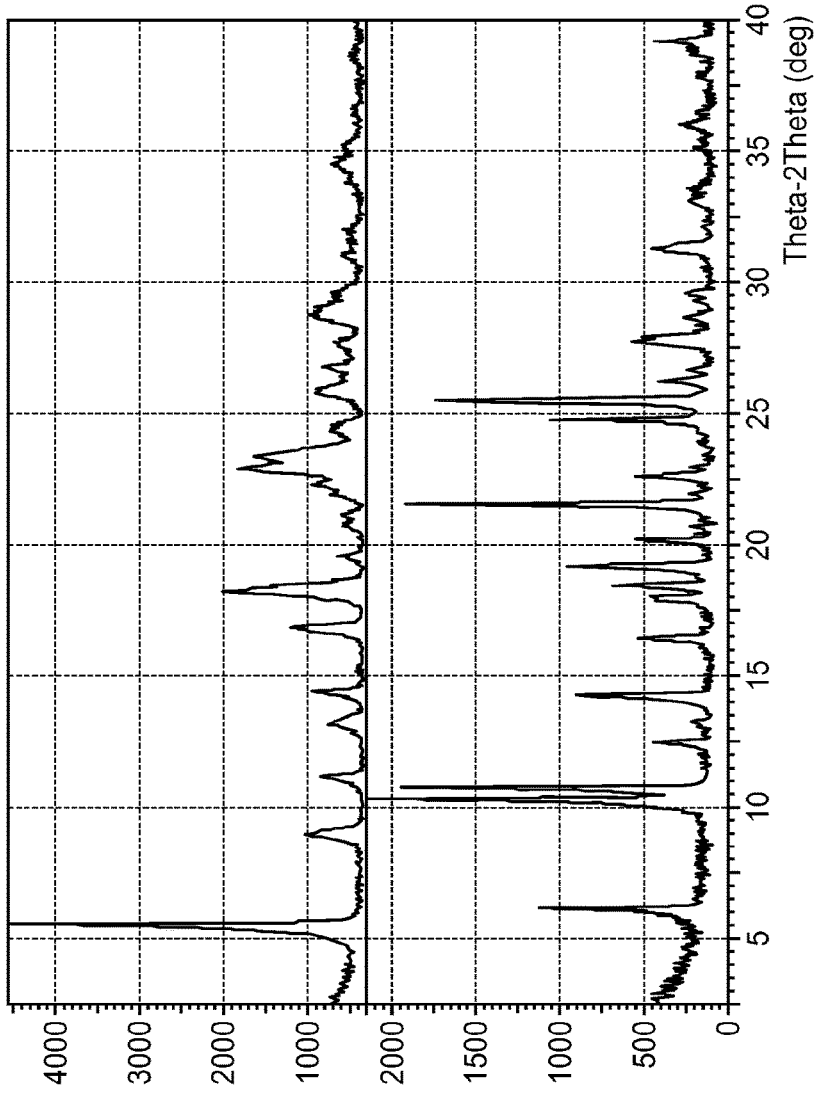

4000
3000
2000
1000
2000
1500
1000
500
0
5
10
15
20
25
30
35
40
Theta-2Theta (deg)

FIG. 5

PROCESS FOR PREPARING AN A2A-ADENOSINE RECEPTOR AGONIST AND ITS POLYMORPHS

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue; a claim printed with strikethrough indicates that the claim was canceled, disclaimed, or held invalid by a prior post-patent action or proceeding.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/333,872, filed Dec. 21, 2011, now U.S. Pat. No. 8,524,883, issued on Sep. 3, 2013, which is a continuation of U.S. patent application Ser. No. 12/765,623, filed Apr. 22, 2010, now U.S. Pat. No. 8,106,183, issued on Jan. 31, 2012, which is a continuation of U.S. patent application Ser. No. 11/701,699, filed Feb. 2, 2007, now U.S. Pat. No. 7,732,595, issued on Jun. 8, 2010, which claims priority to U.S. Provisional Patent Application No. 60/801,857, filed May 18, 2006, and to U.S. Provisional Patent Application No. 60/765,114, filed Feb. 3, 2006, which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to a process for the large scale preparation of an A_{2A}-adenosine receptor agonist, and also relates to polymorphs of that compound, and to methods of isolating a specific polymorph.

BACKGROUND

Adenosine is a naturally occurring nucleoside, which exerts its biological effects by interacting with a family of adenosine receptors known as A_1, A_{2A}, A_{2B}, and A_3, all of which modulate important physiological processes. One of the biological effects of adenosine is to act as a coronary vasodilator; this result being produced by interaction with the A_{2A} adenosine receptor. This effect of adenosine has been found to be useful as an aid to imaging of the heart, where coronary arteries are dilated prior to administration of an imaging agent (for example thallium 201), and thus, by observation of the images thus produced, the presence or absence of coronary artery disease can be determined. The advantage of such a technique is that it avoids the more traditional method of inducing coronary vasodilation by exercise on a treadmill, which is clearly undesirable for a patient that has a coronary disease.

However, administration of adenosine has several disadvantages. Adenosine has a very short half life in humans (less than 10 seconds), and also has all of the effects associated with A_1, A_{2A}, A_{2B}, and A_3 receptor agonism. Thus the use of a selective A_{2A} adenosine receptor agonist would provide a superior method of producing coronary vasodilation, particularly one with a longer half life and few or no side effects.

A class of compounds possessing these desirable properties was disclosed in U.S. Pat. No. 6,403,567, the complete disclosure of which is hereby incorporated by reference. In particular, one compound disclosed in this patent, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, has been shown to be a highly selective A_{2A}-adenosine receptor agonist, and is presently undergoing clinical trials as a coronary vasodilator useful in cardiac imaging.

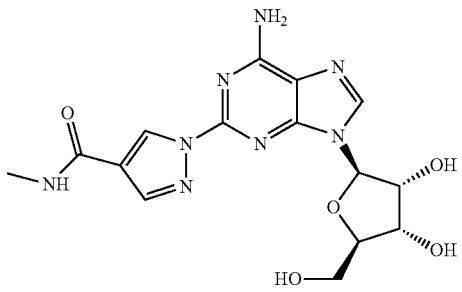
Given the heightened interest in this and similar compounds, it has become desirable to find new methods of synthesis that provide a convenient method for making large quantities of the material in good yield and high purity. The patent that discloses the compound of interest (U.S. Pat. No. 6,403,567) provides several methods for preparing the compound. However, although these methods are suited to small scale syntheses, all synthetic methods disclosed in the patent utilize protecting groups, which is undesirable for large scale syntheses.

Additionally, it was discovered that the desired product (that is (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide) is capable of existing in at least three different crystalline forms, the most stable of which is a monohydrate. This polymorph is stable under relative humidity stress conditions, up to its melting point. Accordingly, it is desirable that the final product produced in the new syntheses is obtained as the stable monohydrate.

SUMMARY OF THE INVENTION

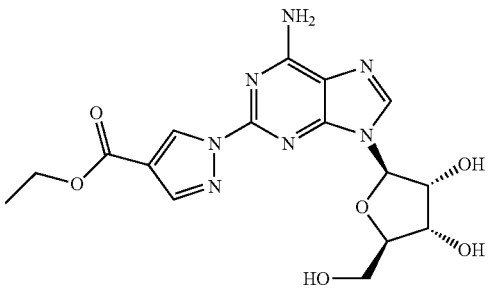
Thus, it is an object of this invention to provide convenient syntheses for the large scale preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, and polymorphs thereof, preferably as its monohydrate. Accordingly, in a first aspect, the invention relates to the preparation of a compound of the Formula I:

Formula I



comprising:
contacting a compound of the formula (3):

(3)



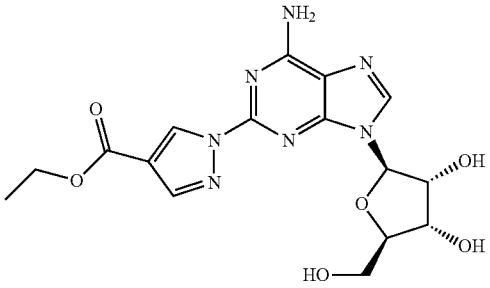
with methylamine.

In one embodiment the reaction is conducted in an aqueous solution of methylamine, initially at a temperature

of about 0-5° C., followed by warming to about 50-70° C. Alternatively, the reaction is conducted as above but in a sealed pressure reactor.

In a second embodiment, the product is isolated as the pure monohydrate by dissolving the product in a solvent, for example dimethylsulfoxide, addition of purified water, filtering the slurry thus formed, washing the contents of the filter with water followed by ethanol, and drying the solid that remains under vacuum at a temperature that does not exceed 40° C.

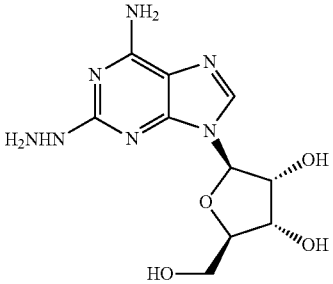
In a second aspect, the invention relates to the preparation of a compound of the formula (3):



(3)

comprising:

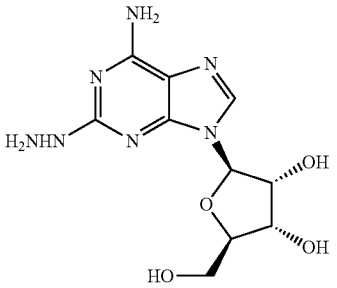
contacting a compound of the formula (2):



with ethyl 2-formyl-3-oxopropionate.

In one embodiment, the reaction is conducted in ethanol, at a temperature of about 80° C., with about 1.1 molar equivalents of ethyl 2-formyl-3-oxopropionate.

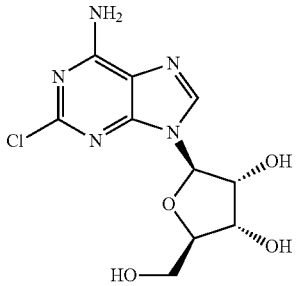
In a third aspect, the invention relates to the preparation of a compound of the formula (2):



(2)

comprising:

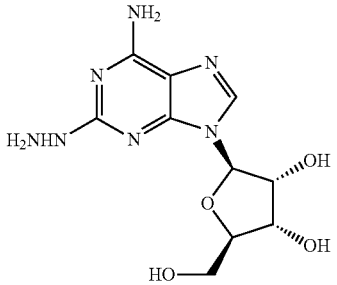
contacting a compound of the formula (1):



(1)

with hydrazine.

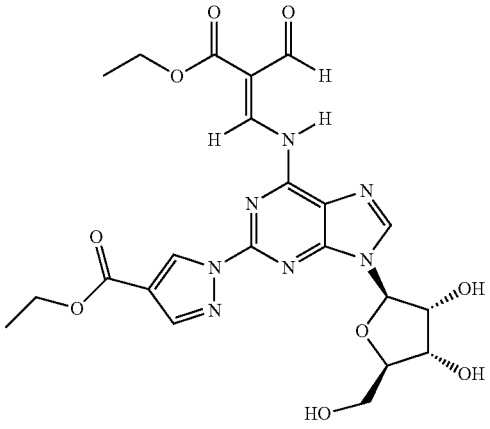
The above described synthesis is suitable for the large scale synthesis of the desired product, which is provided in good yield, although one minor impurity is seen in the final product. This impurity has been shown to be unchanged intermediate of the formula (2); that is, the compound of the formula:



(2)

Although this impurity can be removed from the final product by crystallization, it was decided to seek an alternative synthesis that had all of the advantages of the above synthesis but did not give the compound of formula (2) as an impurity in the final product.

Thus, in a fourth aspect, the invention relates to a method of synthesizing (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide by contacting a compound of the formula (4):



(4)

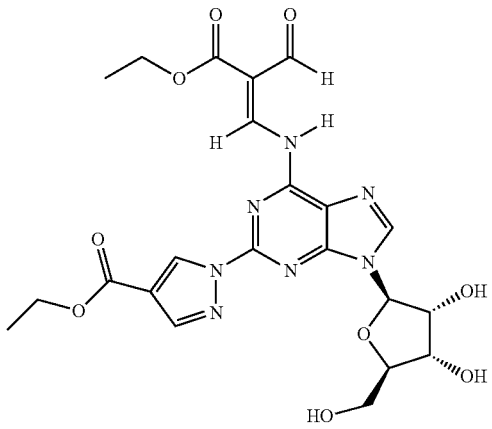
with methylamine.

In one embodiment the reaction is conducted in an aqueous solution of methylamine, initially at a temperature of about 0-5° C., followed by warming to about 50-70° C. Preferably, the reaction is conducted in a sealed pressure reactor.

In a second embodiment, the product is isolated as the pure monohydrate by dissolving the product in a solvent, for example dimethylsulfoxide, addition of purified water, filtering the slurry thus formed, washing the contents of the filter with water followed by ethanol, and drying the solid that remains under vacuum at a temperature that does not exceed 40° C.

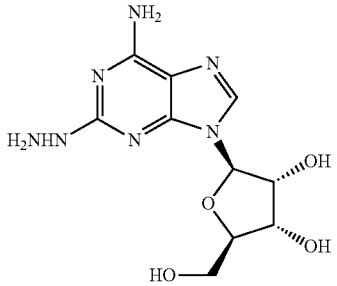
In a fifth aspect, the invention relates to a method of synthesizing a compound of the formula (4):

(4)



comprising contacting a compound of the formula (2):

(2)



with an excess of ethyl 2-formyl-3-oxopropionate, preferably about a 2-10 fold excess, more preferably about a 5-10 fold excess.

In one embodiment, the reaction is conducted in ethanol, at a temperature of about 80° C. The ethyl 2-formyl-3-oxopropionate is present in a 5-10 fold excess.

DEFINITIONS AND GENERAL PARAMETERS

FIG. **1** is a ^{1}H NMR spectrum of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate (Form A).

FIG. **2** shows the thermal analysis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate.

FIG. **3** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate.

FIG. **4** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide Form B.

FIG. **5** shows the X-Ray diffraction pattern for (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide Form C as compared to Form A.

As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not.

The term "therapeutically effective amount" refers to that amount of a compound of Formula I that is sufficient to effect treatment, as defined below, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "treatment" or "treating" means any treatment of a disease in a mammal, including:

(i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;

(ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or

(iii) relieving the disease, that is, causing the regression of clinical symptoms.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "polymorph" is intended to include amorphous and solvates of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide.

It has been discovered that this compound is capable of existing in at least three different crystalline forms, referred to herein as Form A, Form B, Form C, and an amorphous product.

Form A:

This polymorph can be produced by crystallizing 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide from protic solvents, for example ethanol or ethanol/water mixtures, or from a polar solvent, for example dimethylsulfoxide/water. Form A has been shown to be a monohydrate, and is the most stable of the various polymorphs at ambient temperatures. It is stable under relative humidity stress conditions up to its melting point.

Form B:

This polymorph is produced by evaporating under vacuum a solution of 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in trifluoroethanol at ambient temperatures. The X-ray analysis of the crystals was distinctly different from any other polymorph (see FIG. **4**), but it was difficult to determine its constitution, as the X-ray analysis gave disordered broad peaks, and the polymorph contained varying amounts of water. It was found to be difficult to reliably reproduce the preparation of this polymorph.

Form C:

This polymorph is produced by slurrying 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide in acetonitrile for a long period of time at 60° C. The X-ray analysis of the crystals was distinctly different from any other polymorph (see FIG. **5**). Polymorph C was shown to be a variable hydrate, which, at elevated temperatures, desolvates to an unstable form.

Amorphous Material:

This polymorph is produced by heating Form A polymorph at a temperature of up to 200° C. This polymorph is unstable in the presence of atmospheric moisture, forming variable hydrates.

Techniques for Analysis of Forms A, B, C and Amorpheous Material

X-Ray Powder Diffraction

X-ray powder diffraction (XRPD) analyses were carried out on a Shimadzu XRD-6000 X-ray powder diffractometer using Cu Kα radiation. The instrument was equipped with a fine focus X-ray tube, and the tube voltage and amperage were set to 40 kV and 40 mA respectively. The divergence and scattering slits were set at 1" and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A theta-two theta continuous scan at 3°/min (0.4 sec/0.02° step) from 2.5-40° 2θ was used. A silicon standard was used to check the instrument alignment. Data were collected and analyzed using XRD-6000 v.4.1 software.

X-ray powder diffraction (XRPD) analyses were also performed using an Inel XRG-3000 diffractometer equipped with a CPS (Curved Position Sensitive) detector with a 28 range of 120°. The instrument calibration was performed using a silicon reference standard. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. The monochromator slit was set at 5 mm by 80 μm. Samples were placed in an aluminum sample holder with a silicon insert or in glass XRPD-quality capillaries. Each capillary was mounted onto a goniometer head that is motorized to permit spinning of the capillary during data acquisition. Real time data were collected using Cu-Kα radiation at a resolution of 0.03° 20. Typically, data were collected over a period of 300 seconds. Only the data points within the range of 2.5-40° 20 are displayed in the plotted XRPD patterns.

Thermal Analyses

Thermogravimetric (TG) analyses were carried out on a TA Instruments 2050 or 2950 thermogravimetric analyzer. The calibration standards were nickel and Alumel™. Samples were placed in an aluminum sample pan, inserted into the TG furnace, and accurately weighed. The samples were heated in nitrogen at a rate of 10° C./min to either 300 or 350° C. Unless stated otherwise, samples weights were equilibrated at 25° C. in the TGA furnace prior to analysis.

Differential scanning calorimetry (DSC) analyses were carried out on a TA Instruments differential scanning calorimeter 2920. Accurately weighed samples were placed in either crimped pans or hermetically sealed pans that contained a pinhole to allow for pressure release. Each sample was heated under nitrogen at a rate of 10° C./min to either 300 or 350° C. Indium metal was used as the calibration standard. Temperatures were reported at the transition maxima.

Infrared Spectroscopy

Infrared spectra were acquired on Magna 860® Fourier transform infrared (FT-IR) spectrophotometer (Nicolet Instrument Corp.) equipped with an Ever-Glo mid/far IR source, an extended range potassium bromide beamsplitter, and a deuterated triglycine sulfate (DTGS) detector. Unless stated otherwise, a Spectra-Tech, Inc. diffuse reflectance accessory (the Collector™) was used for sampling. Each spectrum represents 256 co-added scans at a spectral resolution of 4 cm^{-1}. Sample preparation for the compound consisted of placing the sample into a microcup and leveling the material with a frosted glass slide. A background data set was acquired with an alignment mirror in place. The spectra represent a ratio of the sample single-beam data set to the background single beam data set. Wavelength calibration of the instrument was performed using polystyrene.

NMR Spectroscopy

Solution phase ^1H NMR spectra of the were acquired at ambient temperature on a Bruker model AM-250 spectrometer operating at 5.87 T (Larmor frequency: 'H=250 MHz). Time-domain data were acquired using a pulse width of 7.5 ps and an acquisition time of 1.6834 second over a spectral window of 5000 Hz. A total of 16,384 data points were collected. A relaxation delay time of 5 seconds was employed between transients. Each data set typically consisted of 128 coaveraged transients. The spectra were processed utilizing GRAMS132 A1 software, version 6.00. The free induction decay (FID) was zero-filled to four times the number of data points and exponentially multiplied with a line-broadening factor of 0.61 Hz prior to Fourier transformation. The 'H spectra were internally referenced to tetramethylsilane (0 ppm) that was added as an internal standard.

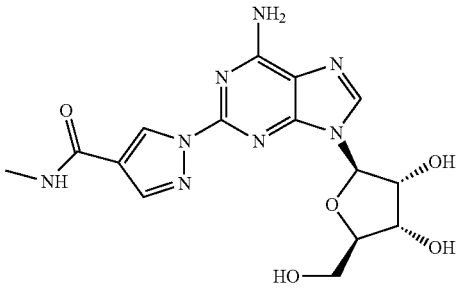
Alternatively, NMR analysis was carried out as described in Example 4.

Moisture Sorption/Desorption Analyses

Moisture sorption/desorption data were collected on a VTI SGA-100 Vapor Sorption Analyzer. Sorption and desorption data were collected over a range of 5% to 95% relative humidity (RK) at 10% RH intervals under a nitrogen purge. Sodium chloride (NaCl) and polyvinyllpyrrolidone (PVP) were used as the calibration standards. Equilibrium criteria used for analysis were less than 0.0100% weight change in 5 minutes, with a maximum equilibration time of 180 minutes if the weight criterion was not met. The plotted data have not been corrected for the initial moisture content.

NOMENCLATURE

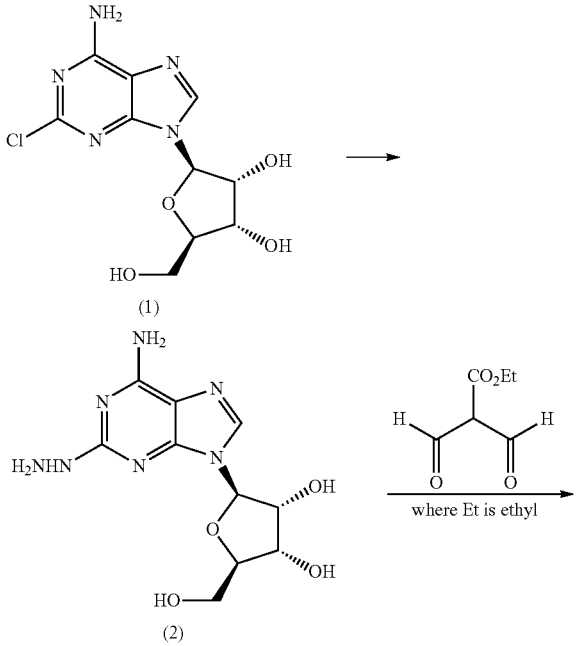
The structure of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is as follows:

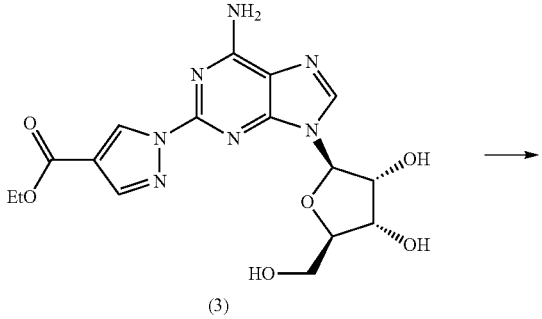


Synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide

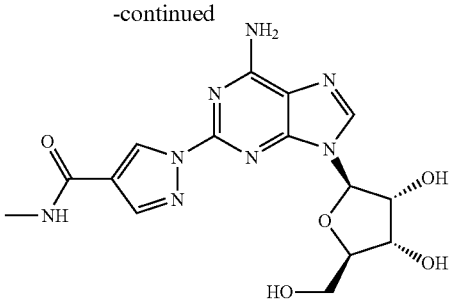
One method for the large scale synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is shown in Reaction Scheme I.

REACTION SCHEME I





-continued



Step 1—Preparation of Formula (2)

The compound of formula (2) is prepared from the compound of formula (1) by reaction with hydrazine monohydrate in the absence of a solvent. The reaction is conducted at a temperature of about 40° C. plus/minus 5° C. When the reaction is complete, the product of formula (2) is isolated by stirring with a protic solvent in which the compound of formula (2) has limited solubility, for example ethanol or isopropanol. The mixture is stirred for about 1-5 hours, and then filtered. The solid is purified by stirring with water, filtering, and washing with water followed by isopropanol and dried under vacuum, which is taken to the next step without purification.

Step 2—Preparation of Formula (3)

The compound of formula (2) is then converted to a compound of formula (3) by reacting with about 1-1.2 molar equivalents of ethyl 2-formyl-3-oxopropionate. The reaction is conducted in a protic solvent, preferably ethanol, at about reflux temperature, for about 2-4 hours. After cooling, to about 0° C., the solid is filtered off, washed with cold ethanol, and dried under reduced pressure. The product of formula (3) is taken to the next step without purification.

Step 3—Preparation of Final Product

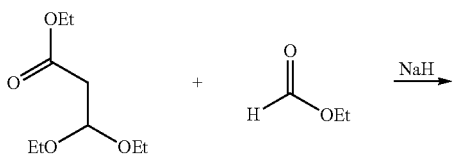
The final product is prepared from the compound of formula (3) by reacting with methylamine, preferably aqueous methylamine. The reaction is carried out at about room temperature, for about 4 hours. The product of Formula I is isolated by conventional means, for example by filtration, washing the solid with cold ethanol, and drying under reduced pressure.

Preparation of Starting Materials

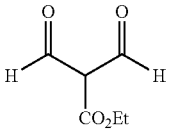
(4S,2R,3R,5R)-2-(6-amino-2-chloropurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol is used as a starting material in step 1. This compound is commercially available.

Ethyl 2-formyl-3-oxopropanoate is used as a starting material in step 2. It is commercially available, or may be made as shown in Reaction Scheme II.

REACTION SCHEME II



-continued

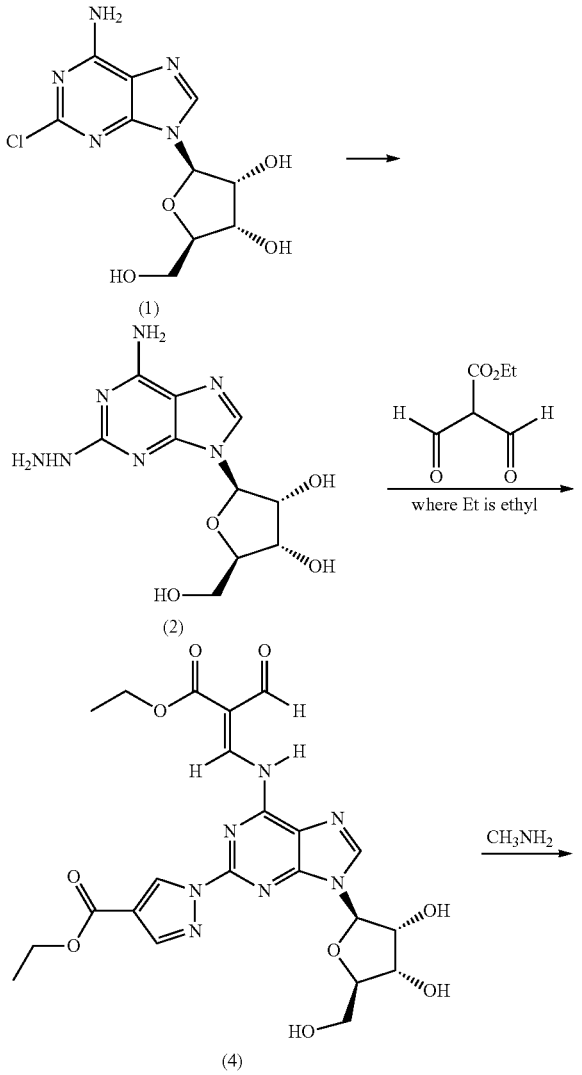


where Et is ethyl

Ethyl 3,3-diethoxypropionate is reacted with ethyl formate in the presence of a strong base, preferably sodium hydride. The reaction is carried out at about 0-5° C., for about 24 hours. The product is isolated by conventional means, for example by the addition of water and extraction of impurities with a conventional solvent, for example t-butylmethyl ether, acidification of the aqueous phase with, for example, hydrochloric acid, followed by extraction with a solvent such as dichloromethane, and removing the solvent from the dried extract under reduced pressure.

A preferred method for the large scale synthesis of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide is shown in Reaction Scheme III.

REACTION SCHEME III



(1)

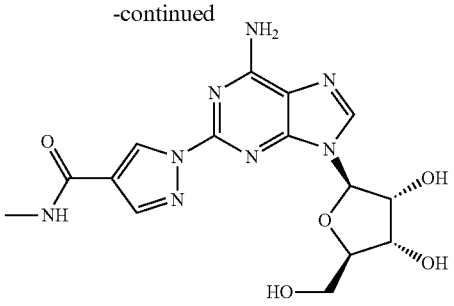
(2)

where Et is ethyl

CH_3NH_2

(4)

-continued



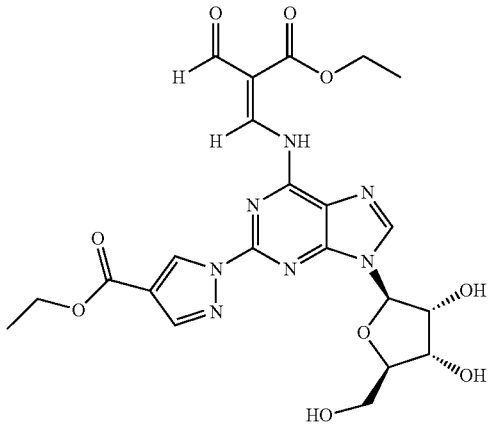
Step 1—Preparation of Formula (2)

The compound of formula (2) is prepared from the compound of formula (1) by reaction with hydrazine monohydrate in the absence of a solvent. The reaction is conducted at a temperature of about 45-55° C. plus/minus 5° C. When the reaction is complete, the product of formula (2) is isolated by stirring with a protic solvent in which the compound of formula (2) has limited solubility, for example ethanol or isopropanol. The mixture is stirred for about 1-5 hours, and then filtered. The solid is purified by stirring with water, filtering, and washing with water followed by ethanol or isopropanol and dried under vacuum, which is taken to the next step without purification.

Step 2—Preparation of Formula (4)

The compound of formula (2) is then converted to a compound of formula (4) by reacting with an excess of ethyl 2-formyl-3-oxopropionate, for example a 2-10 fold excess, preferably about 5-10 fold excess. The reaction is conducted in a protic solvent, for example ethanol, at about reflux temperature, for about 2-4 hours. After cooling, to about 0° C., the solid is filtered off, washed with cold ethanol, and dried under reduced pressure, and the product of formula (4) is taken to the next step without purification.

The compound of formula (4) is drawn as a (2E) alkene derivative, as this is the major isomer formed in this reaction. However, it should be noted that a significant amount of the (2Z) alkene derivative may also be formed in this reaction; that is:



named as ethyl (2Z)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-oxolan-2-yl]-2-[4-(ethoxycarbonyl) pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate.

Accordingly, although the compound of formula (4) is represented as the (2E) alkene derivative only, the term "compound of formula (4)" is intended to include both the

instance where it is solely the (2E) isomer, and the instance where the major portion of the product is the (2E) isomer and a minor portion of the (2Z) isomer is also present. The conversion of the compound of formula (4) to the final product by reaction with methylamine as described in Step 3 proceeds in the same manner whether the compound of formula (4) is present as the (2E) isomer or as a mixture of the (2E) isomer and the (2Z) isomer.

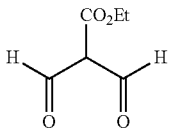
Step 3—Preparation of Final Product

The final product is prepared from the compound of formula (4) by reacting with methylamine, preferably aqueous methylamine. The reaction is initially carried out at about 0-5° C. for about 8 hours, preferably in a pressure reactor, followed by raising the temperature to 50-60° C. over about 1 hour, and maintaining the temperature for 15-30 minutes. The product is isolated by conventional means, for example by cooling to 0-5° C. and maintaining a vacuum for about 1 hour, thus removing the methylamine. The vacuum is removed, and the remaining contents held at 0-5° C. for at least 30 minutes, followed by filtration. The solid thus obtained is washed with water followed by ethanol, and dried under reduced pressure.

This process provides (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate. This polymorph can be further purified by dissolving in dimethylsulfoxide, filtering any solid impurities from the solution, and precipitating the monohydrate from solution by addition of water.

EXAMPLE 1

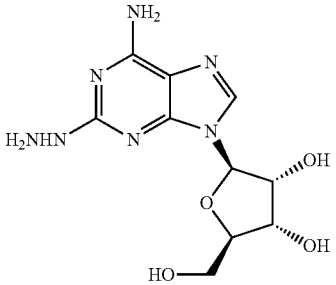
Preparation of Ethyl-2-formyl-3-oxopropionate



A three- or four-neck round bottom flask equipped with magnetic stir bar, thermocouple, digital thermometer, gas inlet and outlet and addition funnel was flushed with argon. Ethyl 3,3-diethoxypropionate (64.5 g) in tetrahydrofuran were charged to the addition funnel. Sodium hydride (21.2 g of a 60% dispersion) was charged to the reaction flask followed by tetrahydrofuran. The contents of the flask were cooled to 0-5° C. in an ice-bath, and ethyl formate (257 g) was added. The mixture was cooled to 0-5° C. and the contents of the addition funnel added dropwise, maintaining an internal temperature of less than 5° C. The ice-bath was removed and the contents allowed to warm to ambient temperature. Consumption of ethyl 3,3-diethoxypropionate was monitored by TLC analysis. The reaction was quenched by addition of ice-water (10.6 vol), and extracted three times with methyl t-butyl ether (5.4 vol each), and the organic layers discarded. The aqueous phase was acidified with conc. hydrochloric acid to a pH of 1 to 1.5. The acidified aqueous layer was extracted three times with dichloromethane and the combined organic layers dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue distilled under vacuum, to provide ethyl 2-formyl-3-oxopropionate, 27.92 g, 70% yield.

EXAMPLE 2

A. Preparation of 2-Hydrazinoadenosine (2)



A flask equipped with a mechanical stirrer, gas inlet, gas outlet and thermocouple was flushed with argon. 2-Chloroadenosine hemihydrate (53.1 g) was added, followed by hydrazine monohydrate (134 g). The mixture was stirred while heating to 40-45° C. for 2 hours. The progress of the reaction was followed by TLC analysis. When the reaction was complete, the heat source was removed and ethanol (800 ml) was added. The mixture was stirred for 2 hours at ambient temperature, then the precipitate collected by filtration. The filter cake was washed with ethanol and dried under reduced pressure for 30 minutes. The solids were transferred to a clean flask equipped with a mechanical stirrer and water (300 ml) was added. The suspension was stirred at room temperature for 18 hours, and the solids isolated by filtration. The filter cake was washed with ice-cold water (300 ml) followed by a wash with ice-cold ethanol (300 ml). The solid was dried under reduced pressure to provide 2-hydrazinoadenosine (41.38 g, 81.4% yield, 99.3% purity).

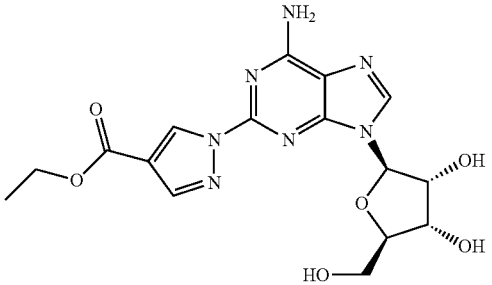
B. Alternative Preparation of 2-Hydrazinoadenosine (2)

A reaction vessel containing hydrazine hydrate (258 g, 250 ml) was heated to 40-50° C. To the warm mixture 2-chloroadenosine hemihydrate (100 g) was added in portions, maintaining the temperature between 45-55° C. The temperature was kept at this temperature for two hours, and then deionized water (500 ml) was added over a period of 30 minutes, maintaining the temperature at 45-55° C. The mixture was then gradually cooled to 0-5° C. over a period of 3 hours, then stirred at this temperature for a further 30 minutes. The solid was then filtered off, and washed with cold (2-5° C.) deionized water (200 ml), followed by ethanol (400 ml). The solid was dried under vacuum for 12 hours, to provide 2-hydrazinoadenosine.

EXAMPLE 3

Preparation of Ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (3)

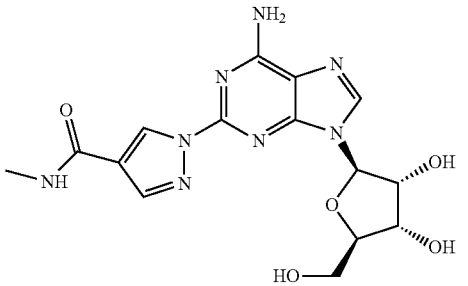
(3)



Ethyl 2-formyl-3-oxopropionate (23.93 g, 0.17 mol) was placed in a flask equipped with mechanical stirrer, gas inlet, gas outlet and reflux condenser. 2-Propanol was added to the flask followed by 2-hydrazinoadenosine (44.45 g, 0.15 mol). The mixture was heated to reflux under stirring for 2-4 hours, following the progress of the reaction by TLC analysis. When the reaction was judged complete, the heat source was removed and the mixture cooled to room temperature. The suspension was cooled under stirring in an ice-bath for 1.5 to 2 hours. The solids were isolated by vacuum filtration, and washed with ice-cold 2-propanol. The product, ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate, was dried under reduced pressure to a constant weight. Yield 54.29 g, purity (by HPLC) 96.6%.

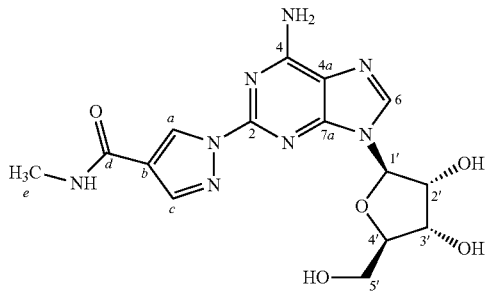
EXAMPLE 4

Preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide



A mixture of ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (46.4 g) and methylamine (40% in water, 600 ml) was stirred at ambient temperature for about 4 hours, following the progress of the reaction by HPLC analysis. The majority of the excess methylamine was removed under reduced pressure, and the remaining mixture cooled at 0° C. for 2 hours. The solid material was filtered off, washed with ice-cold 200 proof ethanol, and dried under reduced pressure, to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate, 36.6 g, purity 99.6%.

The structure of the material was confirmed by ^{1}H NMR (see FIG. **1** and below). Thermal analysis (see FIG. **2**) provided results consistent with the presence of one molecule of water. X-Ray powder diffraction patterns were obtained (FIG. **3**)



^{1}H and ^{13}C NMR spectra were obtained in the following manner. Two samples of the material obtained above were weighed out and dissolved in d_6-DMSO—5.3 mg was used for the ^{1}H spectra, and 20.8 mg was used for ^{13}C spectra. All spectra were acquired at ambient temperature on a JEOL Eclipse$^+$ 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting (Hz)
2	150.5 or 150.3	—	
4	156.4	—	
4a	117.9	—	
6	140.0	8.41	s
7a	150.5 or 150.3	—	
1'	86.9	5.94	D, 6.2
2'	73.7	4.62	m
2'-OH	—	5.50	D, 6.2
3'	70.5	4.17	m
3'-OH	—	5.23	D, 4.7
4'	85.7	3.96	m
5'	61.5	3.67, 3.57	m
5'-OH	—	5.02	D, 5.7
A	140.9	8.07	D, 0.8
B	120.2	—	
C	129.6	8.95	D, 0.8
D	161.7	—	
E	25.6	2.76	D, 4.6
NH_2	—	7.77	br s
NH	—	8.35	Q, 4.6

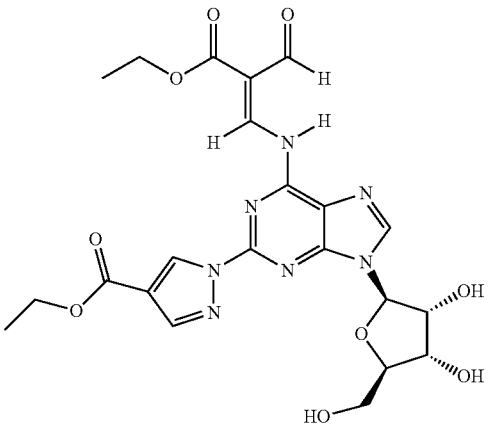
Purification of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate

A solution of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate (100 g) in dimethylsulfoxide (300 ml) was filtered through a 0.6 to 0.8 micron prefilter and a 0.2 micron filter to remove any solid impurities. The filtrate was then slowly added over a period of 1 hour to deionized water (1 liter) with stirring, and the slurry thus produced stirred for not less than 1 hour. The solid was filtered off, washed with deionized water (2×1 liter), and

dried under vacuum for not less than 1 hour. The dried product was then slurried again with deionized water (1.5 liter) for not less than 2 hours, filtered off, and washed with deionized water (1 liter) followed by absolute ethanol (750 ml). The purified product was dried under vacuum at a temperature of not more than 40° C. for not less than 12 hours, to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide monohydrate free of any 2-hydrazinoadenosine impurity.

EXAMPLE 5

Preparation of Ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)-oxolan-2-yl]-2-[4-(ethoxycarbonyl)pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate



A mixture of 2-hydrazinoadenosine (100 g, 0.34 mol), ethyl 2-formyl-3-oxopropionate (242 g, 1.7 mol) and absolute ethanol were charged to a reactor, and the mixture heated to reflux for 2 hours. When the reaction was judged complete, the heat source was removed and the mixture gradually cooled to 5-10° C. over a period of 3 hours. The slurry was stirred for 30 minutes at this temperature, and the mixture filtered. The solid material was washed with cold (5-10° C.) absolute ethanol, and then dried under vacuum at a temperature that did not exceed 40° C., to provide ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-[4-(ethoxycarbonyl)-pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate.

An elemental analysis gave the following results: C, 48.75%; H, 4.86%; N, 18.05%; O, 27.57. Theoretical: C, 49.72%; H, 4.74%; N, 18.45%; O, 27.09. The analysis corresponds within experimental error limits to the hemihydrate of the desired product. (C, 48.89%; H, 4.81%; N, 18.1%; O, 28.12)

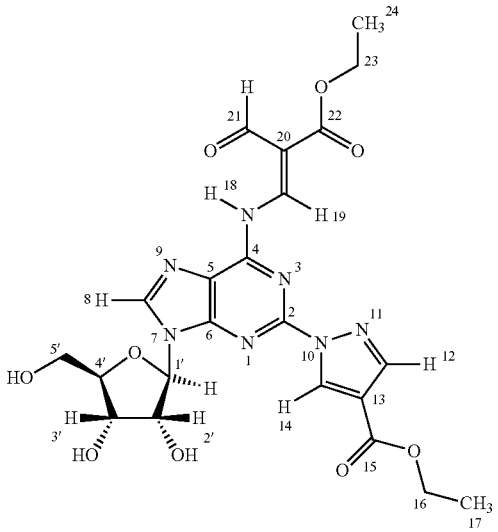
^{1}H and ^{13}C NMR spectra were obtained in the following manner. 20.2 mg of the compound of formula (4) was dissolved in ~0.75 ml of DMSO-d6, and the spectra obtained at ambient temperature on a JEOL ECX-400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts were referenced to the DMSO solvent, 2.50 ppm for ^{1}H and 39.5 ppm for ^{13}C.

Results

The ^{1}H and ^{13}C chemical shifts are listed in Table 1. Two isomers in a ratio of ~60/30 were observed in both the ^{1}H and the ^{13}C spectra, labeled as major and minor in the table.

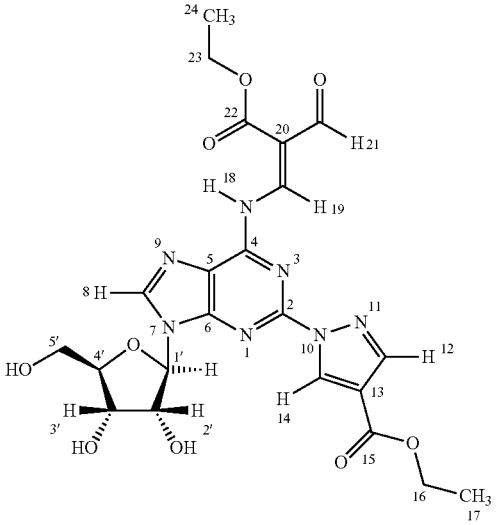
Atom[a]	^{13}C Chemical Shift (ppm)	^{1}H Chemical Shift (ppm)	Multiplicity[b], Splitting (Hz)
21(major)	192.4	9.96	d, 3.6
21(minor)	187.6	9.83	S
22(minor)	167.1	—	—
22(major)	165.2	—	—
15(minor)	161.8	—	
15(major)	161.7	—	—
6(major)	153.1	—	—
6(minor)	152.9	—	—
2(minor)	149.4	—	—
2(major)	149.3	—	—
19(minor)	148.0	9.22	d, 13.0
4(minor)	147.9	—	—
4(major)	147.8	—	—
19(major)	147.5	9.26	d, 12.4, d, 3.6
8(major)	144.9	8.87	s
8(minor)	144.7	8.85	s
12	143.1	8.20-8.23	m
14(minor)	132.8	9.20	d, ~0.7
14(major)	132.6	9.12	d, ~0.7
5(major)	120.7	—	—
5(minor)	120.6	—	—
13	116.7	—	—
20(minor)	107.2	—	—
20(major)	106.1	—	—
1' (major)	87.9	6.07	d, 5.3
1' (minor)	87.9	6.06	d, 5.3
4'	85.8	4.02	q, 3.9
2'(minor)	74.1	4.62	q, ~5.4
2'(major)	74.1	4.61	q, ~5.4
3'	70.1	4.22	q, 4.2
5'	61.0	3.62, 3.73	m
23, 16	60.3-60.8	4.25-4.39	m
17, 24	14.1-14.2	1.28-1.38	m
18(major)	—	12.51	d, 12.4
18(minor)	—	11.47	d, 13.0
2'-OH (major)	—	5.63	d, 6.1
2'-OH (minor)	—	5.62	d, 6.1
3'-OH	—	5.30	d, 5.1
5'-OH	—	5.08	t, 5.5

The compound of formula (4) was confirmed to be a mixture of the following two isomers:



Major

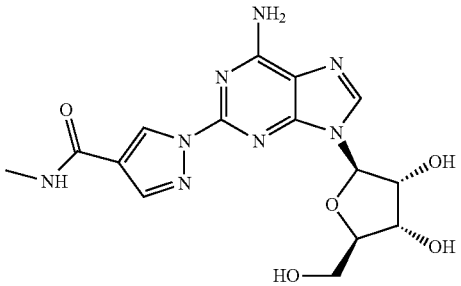
-continued



Minor

EXAMPLE 6

Preparation of (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide from Compound (4)



Aqueous 40% methylamine solution (1300 ml) was placed in a pressure reactor, cooled to 0-5° C., and the product of Example 5 (ethyl (2E)-3-({9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-[4-(ethoxycarbonyl)pyrazolyl]purin-6-yl}amino)-2-formylprop-2-enoate (100 g) added. The mixture was stirred at 0-5° C. for at least 8 hours, monitoring the reaction for completion. When complete, the mixture was warmed, maintaining the temperature between 50 and 60° C. for 1 hour, and then cooled to less than 30° C. over a period of 1 hour. When the temperature was below 30° C., the mixture was degassed using a pressure of 100-150 mm Hg, allowing the temperature to decrease to 0-5° C. The mixture was stirred at 0-5° C. for at least 1 hour, maintaining the pressure at 100-150 mm Hg. The vacuum was then discontinued and replaced by nitrogen, maintaining the temperature at 0-5° C. for not less than 30 minutes. The solid product was then filtered off, washed with water (3×500 ml), then with absolute ethanol (625 ml). The product was dried under vacuum, not allowing the temperature to exceed 40° C., to provide (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide as its monohydrate.

^{1}H and ^{13}C NMR spectra were obtained in the following manner. Two samples of the material obtained above were weighed out and dissolved in d_6DMSO—5.3 mg was used for the ^{1}H spectra, and 20.8 mg was used for ^{13}C spectra. All spectra were acquired at ambient temperature on a JEOL Eclipse$^+$ 400 spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C.

Label	^{13}C shift (ppm)	^{1}H shift (ppm)	Multiplicity, splitting (Hz)
2	150.5 or 150.3	—	
4	156.4	—	
4a	117.9	—	
6	140.0	8.41	s
7a	150.5 or 150.3	—	
1'	86.9	5.94	D, 6.2
2'	73.7	4.62	m
2'-OH	—	5.50	D, 6.2
3'	70.5	4.17	m
3'-OH	—	5.23	D, 4.7
4'	85.7	3.96	m
5'	61.5	3.67, 3.57	m
5'-OH	—	5.02	D, 5.7
A	140.9	8.07	D, 0.8
B	120.2	—	
C	129.6	8.95	D, 0.8
D	161.7	—	
E	25.6	2.76	D, 4.6
NH_2	—	7.77	br s
NH	—	8.35	Q, 4.6

An elemental analysis gave the following results: C, 43.96%; H, 4.94%; N, 27.94. Theoretical: C, 44.12%; H, 4.94%; N, 27.44%; O, 27.09. The analysis corresponds within experimental error limits to the monohydrate.

We claim:

[1. A pharmaceutical composition prepared from a crystalline monohydrate form of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide by adding at least one pharmaceutically acceptable carrier.**]**

[2. The pharmaceutical composition of claim **1**, wherein the monohydrate is substantially free of 2-hydrazinoadenosine.**]**

[3. The pharmaceutical composition of claim **2**, wherein the monohydrate is substantially free of other hydrates or amorphous form.**]**

[4. The pharmaceutical composition of claim **3**, wherein the monohydrate has a purity of at least about 99.6%.**]**

[5. The pharmaceutical composition of claim **4**, wherein the monohydrate exhibits an X-ray powder diffraction pattern having peaks at diffraction angle 2θ (°) of about 6, about 9, about 11, about 13, about 14.5, about 16.5, and about 18 as measured by Cu-Kα1 X-ray powder diffractometry.**]**

6. A pharmaceutical composition of an A_{2A}-adenosine receptor agonist produced by a process comprising the following step:

dissolving a crystalline monohydrate form of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide that is substantially free of 2-hydrazinoadenosine in a pharmaceutically acceptable carrier.

7. The pharmaceutical composition of claim 6, wherein the crystalline monohydrate is substantially free of other hydrates or amorphous forms.

8. The pharmaceutical composition of claim 6, wherein the crystalline monohydrate has a purity of at least about 99.6%.

9. The pharmaceutical composition of claim 6, wherein the crystalline monohydrate exhibits an X-ray powder diffraction pattern having peaks at diffraction angle 2θ (°) of about 6, about 9, about 11, about 13, about 14.5, about 16.5, and about 18 as measured by Cu-Kα1 X-ray powder diffractometry.

10. The pharmaceutical composition of claim 6, wherein the crystalline monohydrate has an X-ray diffraction pattern as shown in FIG. 3.

11. A pharmaceutical composition comprising a crystalline monohydrate form of the compound (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide that is substantially free of 2-hydrazinoadenosine; and a pharmaceutically acceptable carrier.

12. The pharmaceutical composition of claim 11, wherein the crystalline monohydrate is substantially free of other hydrates or amorphous forms.

13. The pharmaceutical composition of claim 11, wherein the crystalline monohydrate has a purity of at least about 99.6%.

14. The pharmaceutical composition of claim 11, wherein the crystalline monohydrate exhibits an X-ray powder diffraction pattern having peaks at diffraction angle 2θ (°) of about 6, about 9, about 11, about 13, about 14.5, about 16.5, and about 18 as measured by Cu-Kα1 X-ray powder diffractometry.

15. The pharmaceutical composition of claim 11, wherein the crystalline monohydrate has an X-ray diffraction pattern as shown in FIG. 3.

16. The pharmaceutical composition of claim 11, wherein the crystalline monohydrate is dissolved in the pharmaceutically acceptable carrier.

17. A pharmaceutical composition comprising 99.6% pure (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide dissolved in a pharmaceutically acceptable carrier.

* * * * *